SURFACE CHANGES DURING CELL DIVISION

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The general features of surface change during cell division have been known for the sea urchin since the inspired work of Dan and his colleagues (Dan, Yanagita and Sugiyama, 1937). Dan's original observations have been substantially confirmed by Hiramoto's excellent analysis (1958). In the present paper we, too, confirm Dan's original observations, although we cannot support his theoretical interpretation (Dan, 1943). The Japanese workers made use of adherent clay or carbon particles to mark the surface; we have used, instead, the echinochrome granules of the egg cortex as natural markers. The detailed behavior of these intensely red granules is of interest because they are fixed in the gelated cortex and there is no possibility of slippage between the surface and the marker. Photographs of the granule patterns give a faithful record of large areas of the cell surface in a way that a few adherent particles can not do. It should be noted that Dan also used the echinochrome granules to mark the movement of the cortex in cleavage but with another aim in view and at much lower magnifications (Dan, 1954). We describe in this paper the surface behavior of a few eggs with some care. The photographs show a "new view" of furrow and pole during division and give especially clear evidence of the polar surface change. The drawings were prepared in every case by tracing photographs.

The term "pole" as used here refers to points on the egg surface normally intersected by the spindle axis. The furrow is considered to have the cell-equatorial plane through its middle. Latitudes are parallel to the equator and longitudes run from pole to pole.

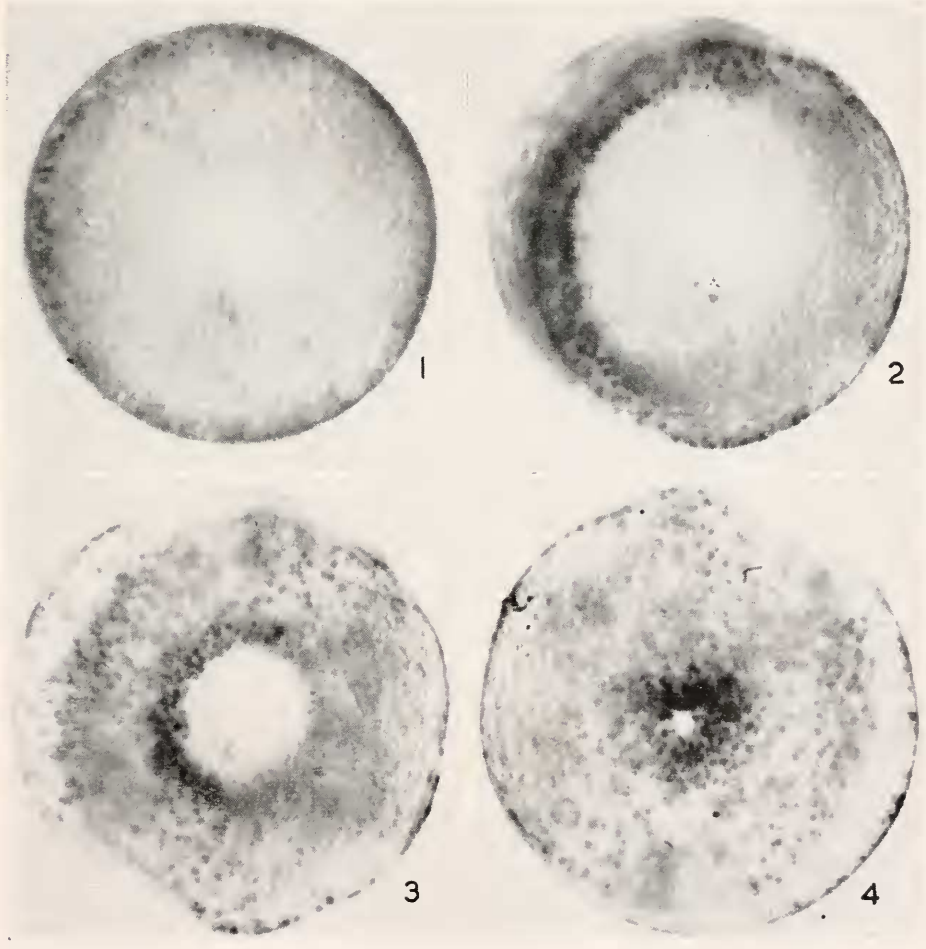
Material and Methods

The red echinochrome granules of the *Arbacia punctulata* egg stand out in strong contrast in the green light of a Wratten No. 58 filter. The individual granules can be photographed sharply through substantial thicknesses of cytoplasm, so that both the surface granules and those deep inside the egg can be seen clearly. The eggs of some females have much darker granules than those of others and, moreover, egg batches differ in the average size of the granules and in the average number of granules per egg.

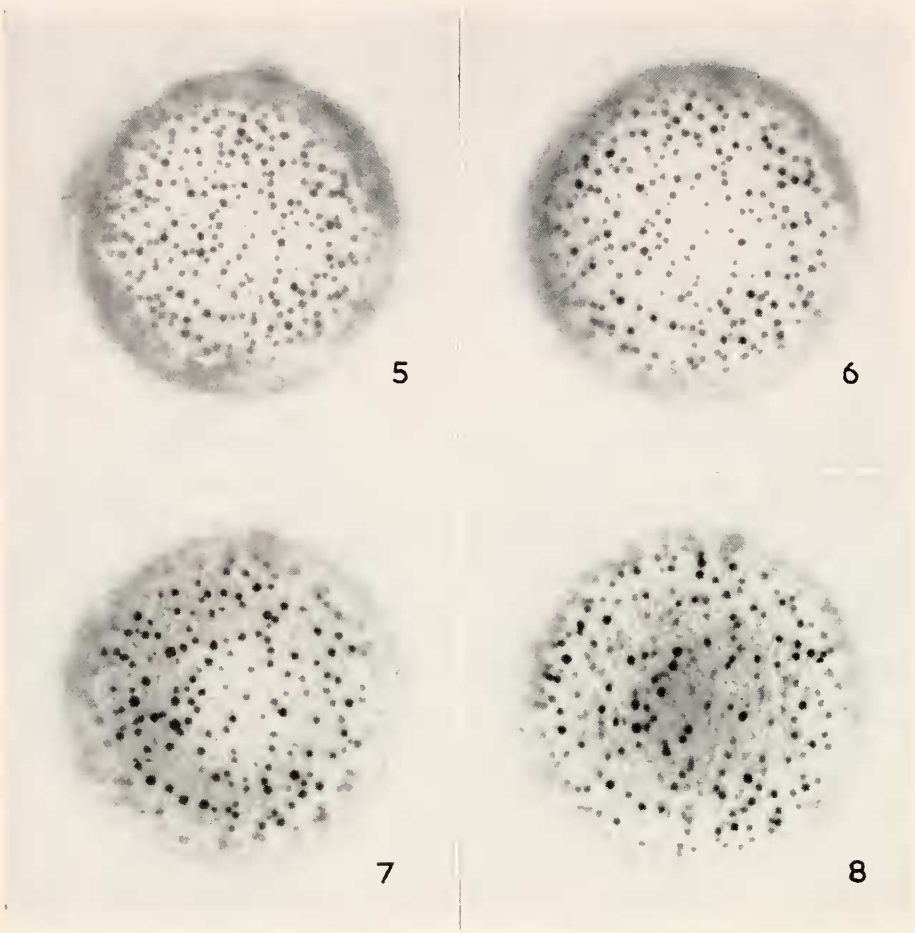
Eggs and sperm were obtained by the electrical method (30 volts A. C.). All of the egg batches used showed at least 95% of the eggs with high fertilization membranes and at least 95% cleavage of the fertilized controls. The fertilization membranes were removed three minutes after insemination by shaking. Just before the cleavage was due, the eggs were transferred to a cold stage held at about 10° C. Cold dry air was blown across the cold stage to prevent condensation; with this apparatus it was possible to take sequential photographs of the same

region of the surface showing the granule patterns in sharp focus. Photographs of the polar surface and views of the furrow seen from the polar axis were taken with the microscope in horizontal position. The eggs were mounted between cover and slide and sealed with Vaseline. When the microscope was tilted into the horizontal position, the eggs came to rest on a glass hair. An occasional cleaving egg was found to be oriented with its long axis coincident with the optical axis. With this technique one polar surface and the equatorial (furrow) plane were observed with good resolution. The inner furrow margin was seen to close like an iris diaphragm, as in Figures 1, 2, 3 and 4. The division of quarter blastomeres can be viewed in this aspect on the upright microscope.

Polyspermic eggs show multiple and intersecting furrows during the first cleavage, in which the regions of furrow intersection show characteristics not found in



FIGURES 1–4. Views of the equatorial plane from the polar axis, showing the iris-like closure of the furrow ring.



FIGURES 5–8. Views of one polar surface of the egg pictured in Figures 1–4. The following pairs of photographs were taken within a few seconds of each other: 1 and 5; 2 and 6; 3 and 7; 4 and 8. Figures 1 and 5, cylinder stage. Figures 4 and 8, at the end of furrowing.

normal, diastral cleavage. Polyspermy was induced by methods detailed previously (Scott, 1946).

RESULTS

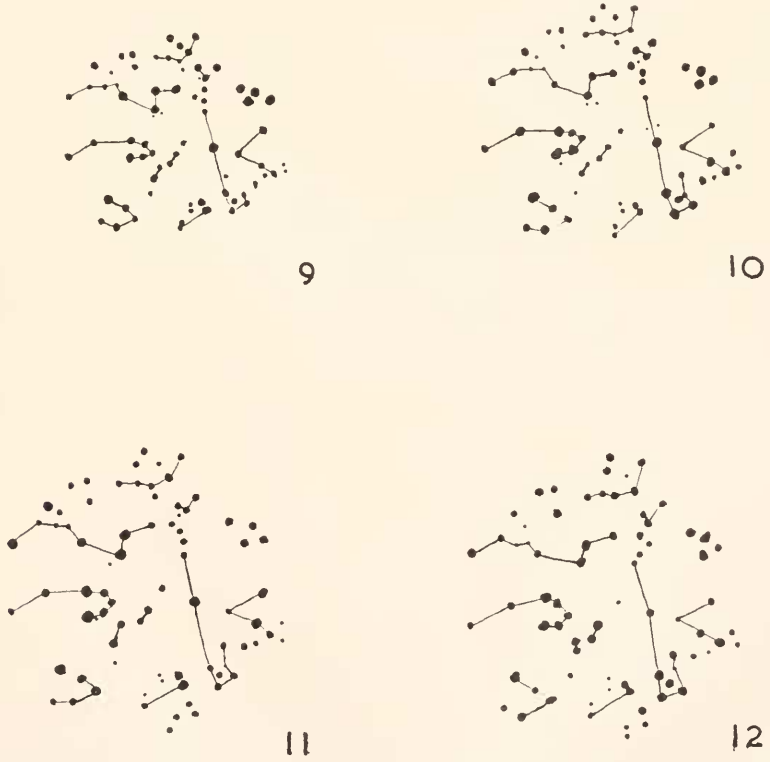
Cortical stiffness indicated by granule patterns

The movements of the echinochrome granules are as follows: (1) In the unfertilized egg they are scattered throughout the cytoplasm; within seven minutes after fertilization at 20° C. most of them have migrated to, and become fixed in, the cortex. (2) The individual granules when observed with oil immersion are seen to undergo a slow random migration within the cortex up until the moment of cell

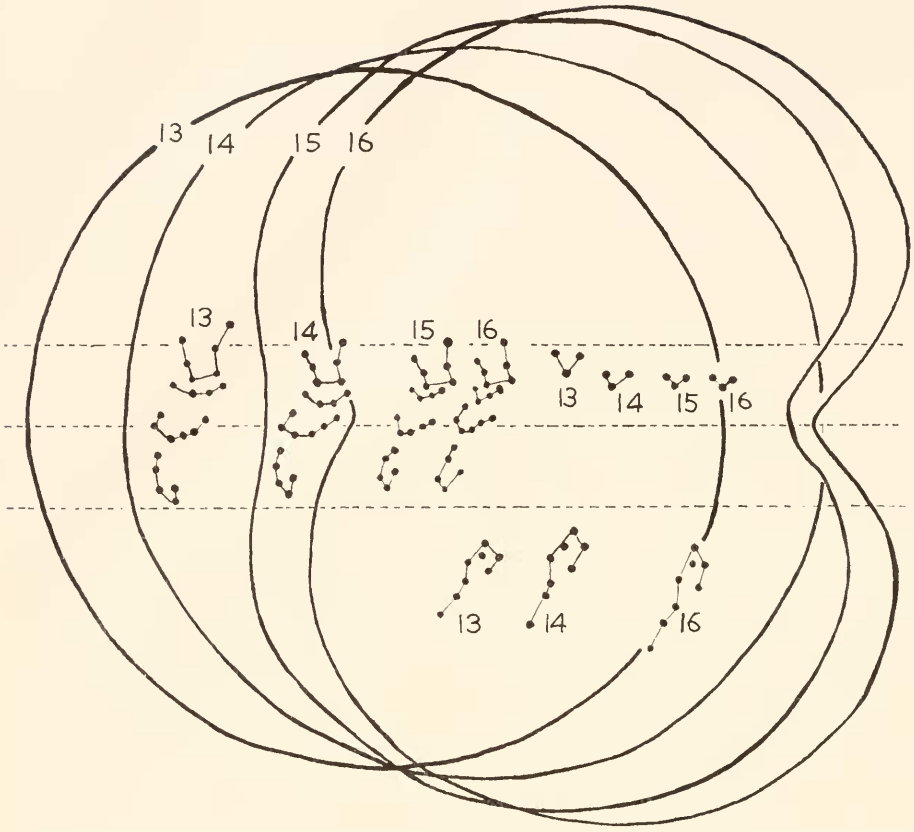
division. (3) During furrowing the cortex is gelated enough to hold the granules in relatively fixed positions so that patterns persist until the end of furrowing when the cortical gel softens and the patterns blur as the granules resume their slow random movement.

Polar surface

The polar surface stretches during furrowing as is illustrated in Figures 5, 6, 7 and 8. The polar surface expands during the first part of the furrowing but remains stretched without further expansion from the time the inner furrow diameter is about 10 micra to the end of the process. The amount of increase in area of the polar zone shown in the photographs is in excess of 65% and the degree of radial expansion is essentially the same for any sector of the polar surface. Constellations of granules have been connected by lines in drawings made from the photographs above (Figs. 9, 10, 11 and 12). In some eggs the expansion continues until the end of furrowing, while in others the expansion is finished by the time the inner furrow diameter reaches ten micra.



FIGURES 9–12. Drawings made from photographs 5–8; the constellations of granules have been connected by lines to make the granule patterns more evident. Polar expansion occurs during the interval between 9–11. There is little change in the polar surface area in late furrowing (compare Figures 11 and 12).



FIGURES 13–16. Egg outlines and corresponding surface granule patterns during four stages of cleavage from the sphere stage to early indenture. Patterns shrink two-dimensionally in Figures 13–14 but only in directions around the furrow in Figures 15 and 16.

Furrow surface

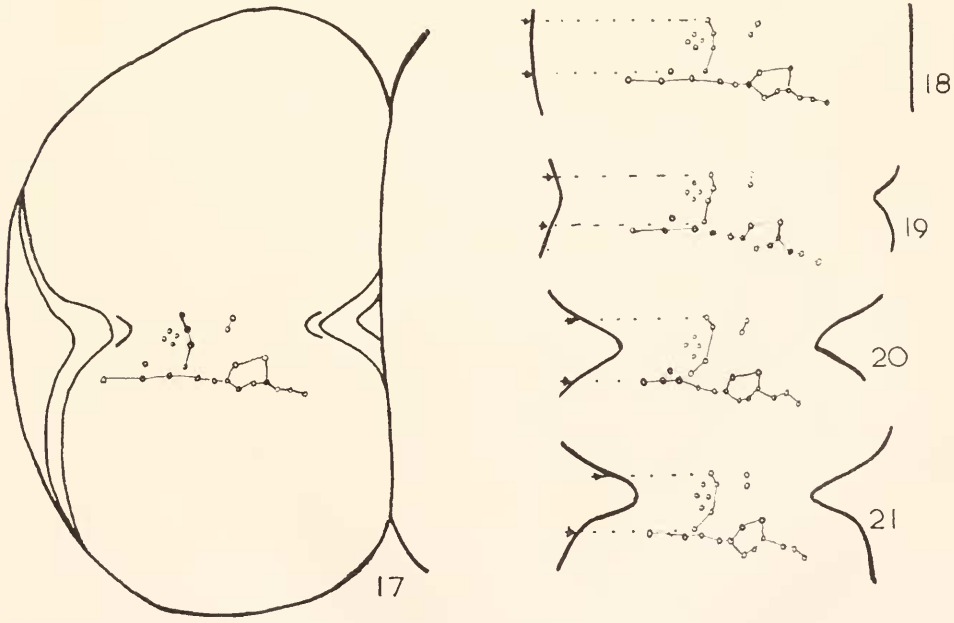
During the earliest phase of cytokinesis the presumptive furrow region flattens around the equator. This is the first phase of surface shrinkage in the potential furrow region. Equatorial shrinkage starts even before cell elongation is apparent. Figure 13 represents an egg just before cleavage is to start. It shows a wide granule pattern on one region of the future furrow surface, while Figure 14 shows the same region a little later when the egg is still nearly spherical. Comparison of Figures 13 and 14 shows that equatorial shrinkage is two-dimensional at this stage in the plane of the surface. The furrow area shrinks along lines of longitude as well as around latitudes. At least one-tenth of the egg surface is surely involved in this shrinkage which represents a band about 15 micra wide around the equator. A longitudinal expansion of the furrow begins at the cylinder stage (Figs. 15 and 22) but its extent can only be appreciated when granule positions are projected to the furrow margin.

Views of the exact center of the furrow show the plastic deformation of the sur-

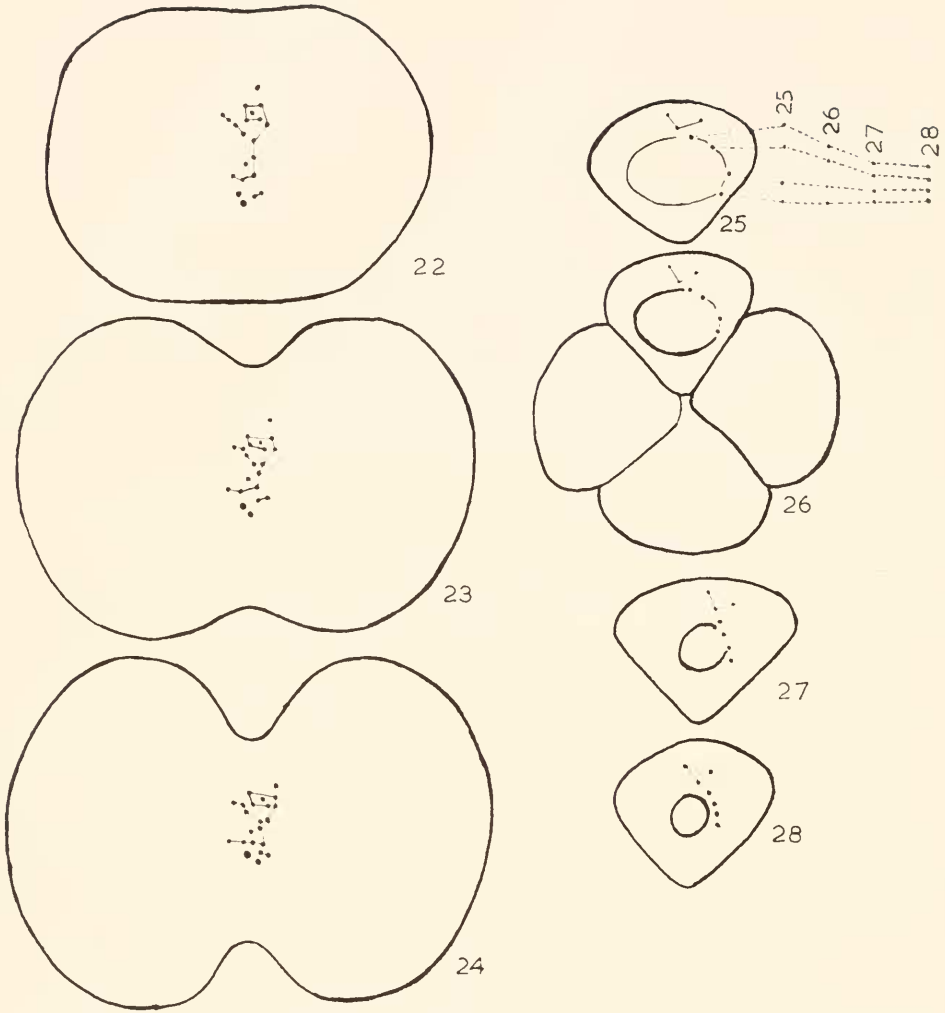
face that occurs in that region. Granule patterns fixed there are deformed by the narrowing equator and by extension in the long axis shown in Figures 17–21. A circumferential shrinkage of the furrow continues throughout cytokinesis, shown also in Figures 17–21, where a row of granules undergoes a steady proportional reduction in length as the cell divides. When viewed from the pole, the furrow closes down like an iris diaphragm, and it is clear that a circumferential shrinkage is occurring throughout this process because granules located on the inner furrow margin are crowded together in a close row as the "iris" boundary moves in. The shrinking circle of granules is clearly seen in Figures 25–28, which illustrate the inner furrow margin in one blastomere during the third cleavage. The successive positions of four granules are diagrammed in four stages of the division. We observe here a shrinkage of this arc to be approximately proportional between each pair of granules. Thus the shrinking substance, whether actively contracting or passively compressed, is shrinking uniformly around the furrow. The granules noted in this case fell behind in the final stages of the furrowing and the furrow peak pushed on beyond them. Shrinkage ends earlier in paraequatorial latitudes than in the equator proper.

Intersecting furrows in polyspermic eggs

Triastral and tetrastral eggs involve intersecting furrows, and at the points of intersection there is a special area of surface whose fate is different from the rest.

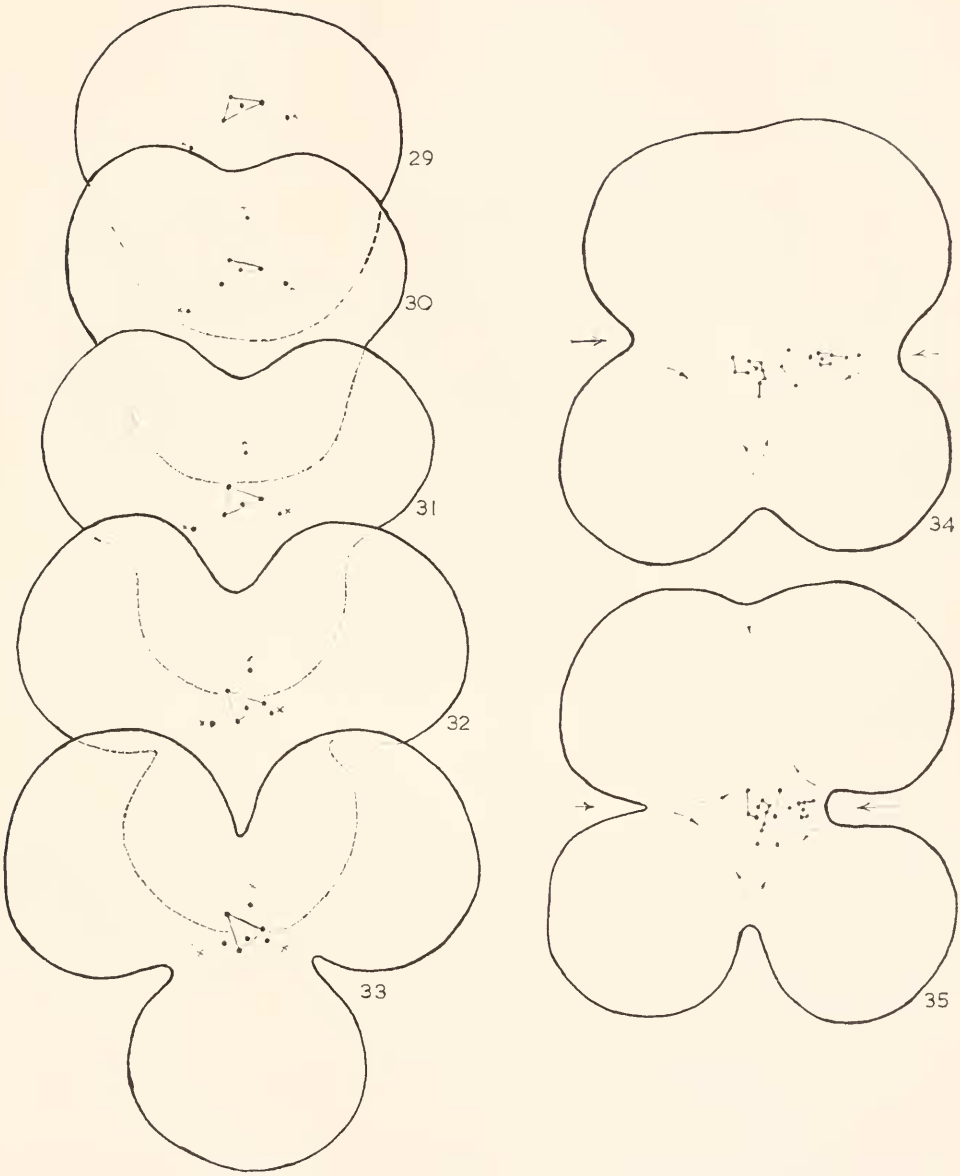


FIGURES 17–21. Granule patterns in mid-furrow and sub-furrow. FIGURE 17. One egg at second cleavage, showing the location of granule patterns in the furrow area. FIGURES 18–21. Details of stages of division in the egg shown in Figure 17, showing circumequatorial shrinkage of patterns on the sub-furrow surface and plastic deformation of patterns at the furrow peak.



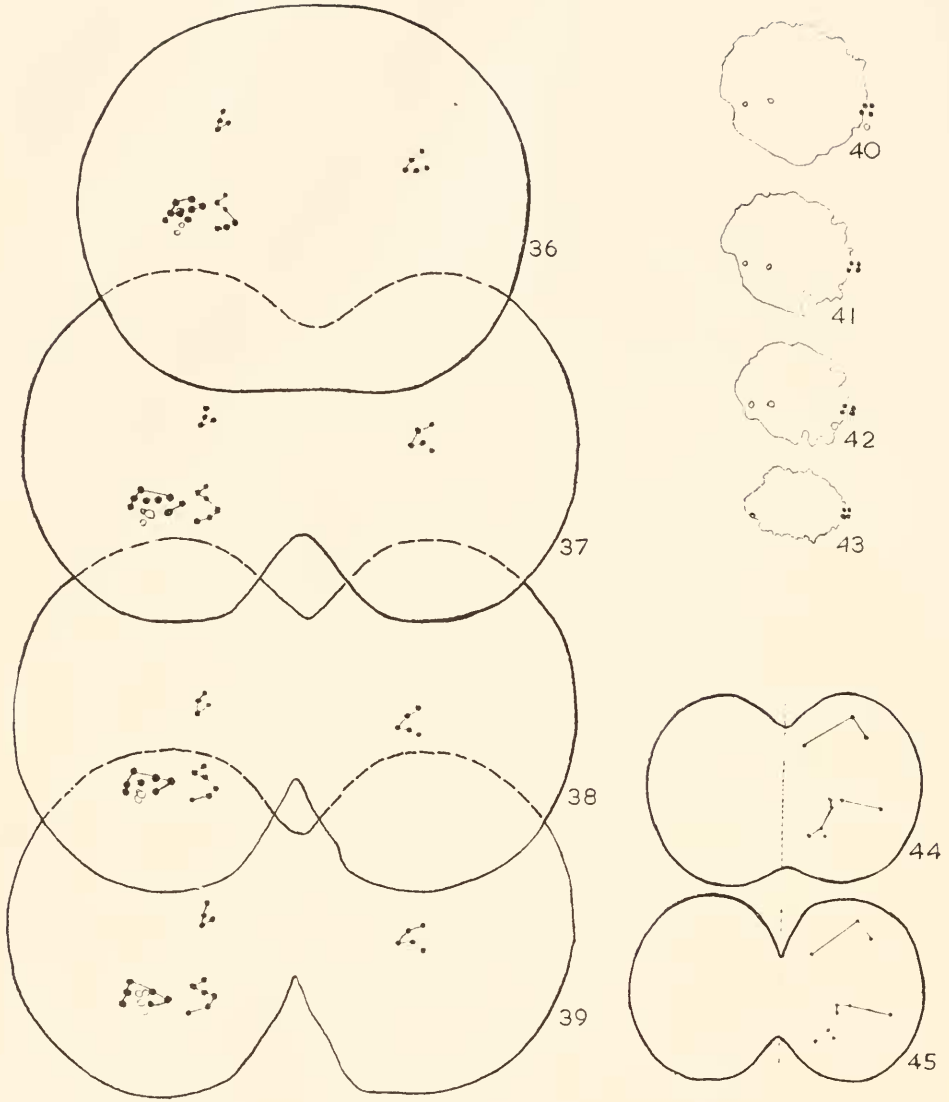
FIGURES 22–28. Granule patterns in the furrow region. FIGURES 22–24. Deformation of granule patterns in the deepening mid-furrow. FIGURES 25–28. View from polar axis, showing iris-like closure of the inner furrow margin of a one-quarter blastomere involving four stages of shrinkage of a pattern of four granules. The relative linear distance between the granules at each stage is illustrated in the numbered diagrams connected by dotted lines.

Such eggs flatten like biscuits as they go off round (Scott, 1946). The flattened surfaces represent regions where the shrinkage is greatest in the initial stage, and vectors of shrinkage occur in more than one direction. They are first marked by concentrations of granules at the animal and vegetal poles. During the formation and the deepening of the multiple furrows, the central island shrinks only in directions tangential to the neck of incipient blastomeres. A triastral cleavage (Figs. 29–33) shows that shrinkage occurs around the blastomere neck so that granules



FIGURES 29–35. Intersecting furrows in polyspermic eggs. FIGURES 29–33. Triastral cleavage. A circumferential shrinkage in the furrows is shown by the movement of granules xxx; the area marked by the triangle does not shrink.

FIGURES 34–35. Tetrastral cleavage. Granule patterns indicate circumferential shrinkage in the dominant furrow and stretching in a direction at right angles.



FIGURES 36–45. Granule patterns on the lateral surface and in the endoplasm. FIGURES 36–39. Lateral surface of the egg, showing the relative immobility of granule patterns. Ishizaka's stationary ring. FIGURES 40–43. Furrow plane and inner furrow margin of an egg in first cleavage; four granules imbedded in the cortex ride on the margin while endoplasmic granules remain motionless. FIGURES 44–45. Endoplasmic granule patterns in a mid-optical section move away from the furrow plane with little distortion.

x-x-x converge but there is a central island, marked by the triangle, which does not shrink, although it might be expected to on the basis of the surface expansion theory (Mitchison, 1952; Swann, 1952). Two stages of a tetrastral cleavage are shown in Figures 34 and 35. In this case one furrow, at the arrow, is in advance of the other and the area of intersection behaves as an integral part of the more advanced furrow. There is marked shrinkage of the surface between the arrows while the "island" surface is, in fact, elongating in the plane of the delayed furrow.

Lateral surface

Ishizaka (1958) has disclosed that two ring areas about the cell can be regarded as stationary if their position during furrowing is related to the center of mass (equator region) and if the spindle axis is fixed. He called them the stationary rings. The position of the rings is determined on two-dimensional outline figures and the term "stationary" refers essentially to the unchanging distance between the ring plane and the equatorial plane on a two-dimensional figure. Now, actually, and Ishizaka realized this, the ring boundaries move further from the equator in a three-dimensional figure when over-the-surface distances are considered. It does not astonish us that the longitudinal expansion of the furrow and of the pole compensate at the latitude of the rings and that they do indeed remain essentially stationary (Figs. 36–39), but while there is little translation of this lateral surface, it does undergo a steady stretching in a longitudinal direction during the whole of the furrowing (see the same Figures 36–39). It results, then, since the lateral surface is relatively fixed, that the subdividing endoplasm within undergoes massive translocation towards one or the other pole.

Endoplasm

A few echinochrome granules remain in the endoplasm and it can be seen that those located in the furrow plane, but outside the spindle, lie quietly in place until the inner furrow margin reaches them (Figs. 40–43). Granules located in a mid-longitudinal plane, away from the furrow, are moved, with some distortion of pattern, further away from the equatorial plane (Figs. 44 and 45). Evidently most of the endoplasm is a deformable plastic mass rather than fluid.

Discussion: Which Theory of Cytokinesis?

Spindle-thrust. In many ways our observations represent a thorough-going confirmation of those made by Dan and his associates. We have substantiated the initial shrinkage of the presumptive furrow. We concur as regards polar, subpolar and subfurrow stretch during furrowing. Yet while we confirm the data, we must reject Dan's spindle-thrust theory. If the 1908 polar abscission experiments of Yatsu on the egg of *Cerebratulus* are not sufficiently conclusive, surely the demonstration that furrowing continues after removal of the amphiaster (Hiramoto, 1956), has made any astral-thrust theory untenable.

Cortical growth. Observations on the newt egg give compelling evidence that a new unpigmented cortex forms in the endoplasm below and ahead of the forming furrow (Selman and Waddington, 1955) and the authors believe that contraction

of the new cortex is a factor in the cell division. It is unlikely that a similar process occurs in the sea urchin egg for the following reasons: (1) all of the equatorial endoplasm is translocated away from the equator as the furrow cuts in; (2) the furrow advances while endoplasm pours through the furrow constriction from one blastomere to the other (in hypotonic calcium-free sea water) (Scott, 1946); (3) cleavage continues smoothly during continual agitation of the furrow endoplasm with a microneedle (Mitchison, 1953).

Surface expansion or furrow contraction. No experiment has yet been designed that will give a clear answer as to whether the active force for cytoplasmic division rests in furrow contraction or in expansion of the non-furrow surface. Our reasons for rejecting the expanding surface theory (Mitchison, 1952; Swann, 1952) are the following: (1) There is now substantial evidence that the furrow cortex has different properties than the non-furrow surface, despite the inability of Mitchison and Swann (1955) to measure a physical difference with their ingenious elastimeter; Beams and King (1937) separated a cleavage-active material from the *Ascaris* egg by ultracentrifugation; Marsland (1950) has demonstrated critical pressure-temperature characteristics of the furrow gel. The furrow cortex has been shown to respond in special ways to hypotonic treatment (Scott, 1946); to dinitrophenol and azide (Kuno-Kojima, 1957). The latter worker has, moreover, isolated fragments of furrow cortex with acid and a detergent. (2) We observed (Scott, 1946) that furrowing may occur in hypotonic sea water with one blastomere increasing in volume while the other is shrinking. This is clearly incompatible with expansion theory. (3) We agree with Marsland and Landau (1954) that surface expansion alone could not provide a vector of force which would direct the surface inward beyond the cylinder stage. (4) The islands noted on trispermic eggs do not undergo continued shrinkage in the way that they would be expected to if active polar expansion were compressing the island from several directions.

In the face of the equivocation still remaining, the contraction theory seems simplest; as Marsland and Landau have noted (1954), it requires no second factor, such as a mechanism for spreading the expansion into the furrow, to explain the "dipping in" after the cylinder stage. What we consider to be strong evidence for the contraction theory was presented in our 1946 paper. It was noted that long stalks develop between blastomeres in hypotonic solutions which, although tenuous, are rigid enough to push the blastomeres 18 micra apart while they continually diminish in diameter.

We can visualize the active furrow surface as a band of specialized cortex about 15 micra wide on the uncleaved egg. We recognize it as different from the rest of the cortex by virtue of the fact that it shrinks, at least in two dimensions, in the initial stage of furrowing. This band of special cortex shrinks most in the center of the furrow and less so on each side, merging into a region which never shrinks at all in the longitudinal direction. Furrow surface shrinkage, which is two-dimensional until the cylinder stage, is circumferential only, around planes parallel to the equator, from the cylinder stage on. The question arises as to why the furrow cortex, previously capable of two-dimensional contraction, is now limited to circumferential contraction around the equator. One might assume that the potentiality for two-dimensional contraction still exists and could

assert itself at any time that resistance could be overcome, but the now cylindrical form and the semi-solid structure allow only the circumfurrow vector of contraction to operate while the longitudinal vector is overcome and the furrow surface expands in the longitudinal direction. In this way we are able to explain the oriented contraction without assuming a pre-orientation of contractable micelles around the equator.

We consider that our observations are in substantial agreement with the contraction hypothesis as formulated by Marsland and Landau (1954). In one detail, however, we can not support their theoretical views. According to their hypothesis, the cortical gel at the furrow peak undergoes solation, with the result that subfurow cortex moves into position at the furrow peak. The authors put it as follows (Marsland and Landau, 1954, p. 532): "Thus more and more cortical gel, possessing an unexpended fund of contractile energy is brought into an operative position as the furrow deepens. Therefore, in the final stages of furrowing, to complete the cleavage it is only necessary to assume that the region of active contraction shifts from the trough of the furrow to the side-walls, first to the region immediately adjacent to the trough and later, to a more peripheral site, somewhat removed from the trough (Stages 4 and 5). In this way, the gel at the very bottom of the trough, having performed its contractile function, could undergo solation, clearing the way for the approach and final fusion of the cell membrane, which severs the stalk between the daughter cells." We have observed, however, that the echinochrome granules are scattered throughout the thickness of the cortex; we have seen no evidence of a non-granular under-layer, which, if present, might undergo solation. Furthermore, as far as we can tell, no furrow granules are liberated to the endoplasm during division—either at the furrow peak or elsewhere. What one observes in fact, is a steady, circumferential contraction of the furrow cortex from the beginning to the end of the process; the cortex changes its shape exceedingly but maintains its integrity throughout. It is possible that new material possessing contractile capacity is added *among* the granules of the furrow, and that the material whose contractile power has been exhausted is expelled, but the basic fabric of the furrow cortex is not destroyed and in any case no granules escape to the endoplasm.

Summary

1. A study of cell surface changes during cell division has been made, using echinochrome granules as natural markers rather than adherent bits of clay or dye.

2. The polar surface of the sea urchin egg stretches symmetrically in all radii from the pole during the first two-thirds of furrowing, and either remains at maximum stretch or shrinks somewhat during the latter third.

3. The furrow surface shrinks in two dimensions in the plane of the surface during earliest furrowing to the cylinder stage; it shrinks latitudinally in a degree which is proportional to the distance from the equator and it expands longitudinally from the cylinder stage on; the cortex undergoes plastic deformation at the peak of the furrow; there is no liberation of cortical echinochrome granules to the endoplasm at any stage of division.

4. All of the cortex is relatively rigid throughout division. The first signs of softening occur at the poles during late furrowing.

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